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PRINCIPLES OF BIOCHEMISTRY: General Aspects

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in the structure of the molecule and the receptor itself; this is analogous to the formation of an enzyme-substrate complex. The second function is activation of specific biological processes.

Receptors may be studied in several ways. The conventional approach has been to define the characteristics of receptors by an examination of some consequence of the receptor interaction. For example, one can characterize hormone receptors by the nature and intensity of a specific physiological effect induced by a series of structurally related hormones or drugs (Chap. M11). Such studies describe the specificity of the hormone receptor, that is, the molecular structural features necessary for interaction with that particular receptor. Although such studies have provided much information about receptors, they are indirect. They describe the receptor (operationally defined as the first step in the sequence of hormone-stimulated events) in terms of what is perhaps the most remote event in the sequence that leads to a specific physiological effect. An unknown number of steps must intervene between the hormone-receptor interaction and the effect which is being measured. More recently, techniques for studying directly the binding of radioactively labeled hormones, drugs, and other substances to their specific receptors have been widely applied. In a few cases the receptor macromolecules have been isolated and characterized.

As noted above, many kinds of substances interact with specific receptors. For the purposes of this discussion, all such agents will be referred to as *ligands,* viz., an ion or molecule that binds with a high degree of specificity to a macromolecule.

The interaction of a ligand (L) with its receptor can be described in terms of its *affinity* and *activity*. The affinity of binding reflects the energy involved in the formation of the ligand receptor (LR) complex and can be directly assessed. It is also reflected in the concentration range over which the ligand elicits a graded physiological response. The remarkable affinities of many hormone-receptor interactions are exemplified by the fact that plasma concentrations of hormones are often in the range of 10^{-11} to 10^{-9} M (Chap. M11). Yet, even at these very low concentrations the agent binds specifically to its receptors. The second property of ligand interaction with receptors concerns the nature and intensity of the evoked physiological response, which is the biological activity of the ligand. This latter property is dealt with in Chap. M12.

Ligand-Receptor Interactions

Ligand-Receptor Binding The ligand-receptor interaction can be measured by radioligand binding studies or, somewhat less directly, by examination of dose-response curves for a biological effect (Chap. M12). The basic requirements for such studies are a radiolabeled form of the ligand and a source of receptors, such as a membrane fraction, intact cells, or solubilized preparations. The receptors and the ligand are incubated until equilibrium is reached, and the amount of labeled ligand bound to the receptors is determined. When particulate preparations are used, this is generally accomplished by millipore filtration, centrifugation, or equilibrium dialysis, which separates free from receptor-bound ligand. With soluble preparations receptor-bound and free ligand may be sep-

arated by gel filtration, equilibrium dialysis, precipitation of the receptor-bound ligand by polyethylene glycol, differential adsorption of free labeled hormone to talc or charcoal, or other methods.

Criteria for Receptor Identification In general, several criteria must be met if true receptors are being labeled; the most important are the following:

1. Saturability There should be only a finite number of receptor sites; thus the receptor binding phenomenon should display saturability. Most cells contain only a few hundred to a few thousand receptors of a particular type. In contrast, "nonspecific" binding is that binding of a radioligand which occurs to various nonreceptor sites. It is generally nonsaturable and linearly related to free ligand concentration.

2. Affinity The concentration range over which the ligand occupies the receptors should be comparable to the range over which a biological response is elicited. For reasons discussed later (Chap. M12) the affinity of a ligand for its receptor assessed by direct binding studies may not agree exactly with the apparent affinity determined from a biological dose-response curve.

3. Specificity This is the most important criterion, since specificity is the hallmark by which receptors are defined. The details of specificity apparent from the ability of various ligands to stimulate (or inhibit) a biological response presumably reflect the specificity of binding of ligand and receptor. Thus the sites labeled by a radioligand should exhibit the specificity and stereospecificity of the biological response mediated by the receptor. For example, the order of potency of a series of hormones or hormone analogs in eliciting a biological response should be exactly reflected in their order of potency in competing for the binding sites. Any significant discrepancy in such potency series would raise questions as to the nature of the sites being labeled. It should be noted that in addition to physiological receptors, other biological macromolecules can potentially bind ligands with high affinity. These include transport proteins and degrading enzymes. If such proteins are labeled, however, their specificity would differ from that of the physiological receptors.

For some receptors, e.g., those for hormones or neurotransmitters, ligand binding studies *alone* cannot prove that a particular binding site is *equivalent* to a physiological receptor. This is because, in addition to their ability to bind ligands, receptors also transduce this binding into a biological signal. Thus the ultimate proof that an isolated binding site is a receptor requires the successful reconstitution of the hormonally responsive system from the isolated components. This means that when the isolated binding site is recombined with other biochemical effector components (e.g., enzymes, transport systems, etc.), it should convey upon the effector component sensitivity to the appropriate ligands. Such reconstitutions have been achieved in only a few systems.

Law of Mass Action and Graphical Analysis of Ligand Binding In receptor binding studies, the simplest assumption concerning the interaction of a radioactive ligand L with a receptor R is the formation of a complex RL, as shown in Eq. 1 for a bimolecular reaction:

$$L + R \rightleftharpoons RL \qquad (1)$$

At equilibrium,

$$\frac{[R][L]}{[RL]} = K_L \tag{2}$$

where K_L is the dissociation constant of the complex and [R], [L], and [RL] represent the concentrations of free receptor, free ligand, and receptor-bound ligand, respectively. Since the total concentration of receptors $[R_t]$ equals [R] + [RL], Eq. 2 may be rewritten as

$$\frac{[R_t - RL][L]}{[RL]} = K_L \tag{3}$$

which rearranges to

$$\frac{[RL]}{[R_t]} = \frac{[L]}{K_L + [L]} \tag{4}$$

The ratio $[RL]/[R_t]$ is the fraction of total receptors occupied by ligand. At one-half maximal occupancy of the receptors, $[RL]/[R_t] = 0.5$ and $K_L = [L]$. Thus, in the simplest case the concentration of ligand required for half maximal occupancy of the receptors is equal to K_L.

Equation 4 can be further rearranged to give

$$[RL] = \frac{[R_t][L]}{K_L + [L]} \tag{5}$$

This is the familiar hyperbolic function in which [RL] approaches $[R_t]$ as [L] becomes large. In radioligand binding studies Eq. 5 is useful for determining the number of receptor binding sites R_t and their affinity K_L for the ligand L. Experimentally, the radioligand is added over a range of concentrations to a fixed concentration of receptors, present in a membrane fraction, cell suspension, or in soluble or purified form. The level of binding which is approached asymptotically at high ligand concentrations is R_t, and the concentration of free ligand which fills half the receptors $[R_t]/2$ represents the K_L. The relationship is plotted in Fig. 14.1*a*. Alternatively, this relationship may be plotted as $[RL]/[R_t]$ vs. log [L] (Fig. 14.1*b*). This plot is a symmetrical sigmoidal curve that has a maximal slope at the midpoint of 0.58 per tenfold change in concentration.

Plots such as that shown in Fig. 14.1*a* are often used in hormone receptor research to determine K_L and R_t; however, the horizontal asymptote is difficult to determine with accuracy. Accordingly, several linearized forms of such binding curves are generally used. An example is the Scatchard plot (Fig. 14.2*a*). Equation 3 can be rearranged as follows:

$$\frac{[RL]}{[L]} = \frac{([R_t] - [RL])}{K_L} \tag{6}$$

Let us redefine [RL] as bound ligand or B, [L] as free ligand or F, and R_t as B_{max}, and then Eq. 6 becomes

$$\frac{B}{F} = \frac{B_{max} - B}{K_L} \tag{7}$$

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